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Short communication

COLICIN E2 PRODUCING BACTERIA FROM YARE RIVER

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ABSTRACT: Colicins are a group of specific, plasmid (Col-factor) mediated bacteriocins produced by different strains of *E. coli* and related species. In this study, a bacterial colony, *E. coli* NY12 strain, recovered from the Yare River, England demonstrated to produce an E colicin. This E colicin was further shown to be located on a plasmid which was subsequently transferred to *E. coli* JM83 *hsd*R. The JM83 derivative, which expresses the transferred ColE plasmid was then tested against nine different E colicin producing strains which specify nine different immunity groups. The result displayed that the E colicin produced by NY12 strain define the same immunity group as E2, which is, like all known E colicins, encoded on a plasmid and it was named pColE2-AT. Plasmid analysis and gel electrophoresis confirmed that pColE2-AT was smaller than pColE2-P9. Studies on the recombinant DNA, pAT11 and pAT12 revealed that the E colicin operon, set of genes, the structural, immunity, and lysis genes of pColE2-AT resides within the same *Eco*RI fragment.

Key words/phrases: ColE-factor, E colicin operon, recombinant plasmid, transformation

INTRODUCTION

Bacteriocins are usually plasmid encoded complex protein substances produced by various bacteria against closely related species. However, bacteriocin

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production is associated with suicidal consequences in that the bacteriocin produced against cohabiting and closely related species ensues the lysis and death of the producing cell itself. E colicins are plasmid-coded bacteriocins which use the same receptor, the gene product of *E. coli* chromosomal gene *btuB*, to bind to sensitive *E. coli* cells (Chak and James, 1984). Each E colicin plasmid encodes a colicin structural gene, an immunity gene and a lysis/release gene in operon (Chak and James, 1984; 1986; Cooper *et al.*, 1986). Immunity protein protects the colicin producing cell against colicins of the same immunity type. Based on the immunity test, E colicins may be divided into colicin E1 to E9 (Cooper and James, 1984; Curtis *et al.*, 1989). E colicin production is controlled and regulated by a set of genes on the plasmid forming an " SOS " response operon. Colicin production is induced by the damage of the host cell DNA, which can be induced by mitomycin C or UV irradiation. The synthesis of immunity gene protein appears to be constitutive (Chak *et al.*, 1991). The lysis gene is promoterless and its transcription depend upon transcriptional read through from the strong promoter of colicin structural gene (Chak and James, 1985).

In this study, a bacterial colony (*E. coli* NY12 strain) recovered from Yare River Norwich, England was established to carry ColE plasmid. The E colicin produced by this strain was further classified to a specific immunity group on the basis of its activity and/or immunity against known standard E colicin producing strains.

MATERIALS AND METHODS

Bacterial strains

E. coli JM83 *hsd*R, a restriction-deficient derivatives of *E. coli* JM83 ara$^+$ lac$^-$ pro$^-$ thi$^-$ rpsL ϕ80dLacZM15 was the host for transformation. Derivatives of *E.coli* W3110 were BZB2014 (pColE1-K53), BZB2125 (pColE2-P9), BZB2149 (pAPBZ101 (E3)), BZB2107 (pColE4-K260), BZB2108 (pColE5-099), BZB2109 (pColE6-CT14), BZB2110 (pColE7-K317), BZB2102 (pColB-K260) and BZB2103 (pColD-CA23). *E. coli* JM103 ColE9 (pMC27), *E. coli* JM103 ColE8 (pRS38), *E. coli btu*B$^-$ and *K. pneumoniae* UNF5023. The cloning vector pUC18, 2686 bp (amp^R lacZ) with a polylinker insertion site was used for

recombinant DNA preparation (Chak *et al.*, 1991). Source of all bacterial strains and plasmids was from the collections of Dr. R. James.

Bacteriocin production and classification

Colicin production and immunity tests were done as described by Cooper and James (1984).

Preparation of E colicin extracts

Colicin extracts from cultures of *E. coli* NY12 and *E. coli* pColE2-P9 were prepared as shown by Chack and James (1984).

Plasmid isolation, restriction, ligation, transformation and gel electrophoresis

These were done as described in Curtis *et al.* (1989).

RESULTS AND DISCUSSION

From 105 bacterial colonies recovered from Yare River, Norwich, England, only 11 were shown to inhibit the growth of one or both test strains (data not shown). The bacterial strain, *E. coli* NY12, was demonstrated to be active only against *E. coli* JM103, but not against its *btu*B$^-$ mutant or against *K. pneumoniae* UNF5023 (Table 1). The result confirmed that NY12 strain produced a bacteriocin which is a member of the E colicin family but not other colicins or klebicin. This is because of the fact that colicin B and colicin D are both known to be active against *E. coli* and *Klebsiella spp.* but E colicins are active only against *E. coli* (James, 1988).

On the basis of colicin/immunity test with known standard colicin E (E1 to E9) producers of *E. coli* W3110 and JM103 strains, it was established that NY12 strain produced colicin E2 (Table 1). The plasmid preparations of *E.coli* NY12 and *E.coli* W3110 (pColE2-P9) transformed competent JM83 cells into E colicin producer. This result demonstrated that the colicin E2 produced by NY12 strain was encoded on a plasmid (Fig. 1, lane f, which was named as pColE2-AT) like all known E colicins (Chak *et al.*, 1991). Results of the activity/immunity pattern and gel electrophoresis of *E. coli* JM83 transformants with the plasmid

preparations of pColE2-AT and pColE2-P9 displayed the same activity/immunity pattern as their original hosts (Table 2).

Table 1. Classification of colicin produced by *E. coli* NY12 strain.

Colicin producing bacteria	Test bacteria (*E. coli*)												
	JM103	*btu*B⁻	NY12	E1	E2	E3	E4	E5	E6	E7	E8	E9	*K. pneumoniae* UNF5023
ColE1	+	-	+	-	+	+	+	+	+	+	+	+	-
ColE2	+	-	-	+	-	+	+	+	+	+	+	+	-
ColE3	+	-	+	+	+	-	+	+	+	+	+	+	-
ColE4	+	-	+	+	+	+	-	+	+	+	+	+	-
ColE5	+	-	+	+	+	+	+	-	+	+	+	+	-
ColE6	+	-	+	+	+	+	+	+	-	+	+	+	-
ColE7	+	-	+	+	+	+	+	+	+	-	+	+	-
ColE8	+	-	+	+	+	+	+	+	+	+	-	+	-
ColE9	+	-	+	+	+	+	+	+	+	+	+	-	-
ColB	+	+	+	+	+	+	+	+	+	+	+	+	+
ColD	+	+	+	+	+	+	+	+	+	+	+	+	+
NY12	+	-	-	+	-	+	+	+	+	+	+	+	-

+, lysis zone formation; -, no lysis zone formation.

Table 2. The activity/immunity pattern of *E. coli* JM83 transformant carrying original col-factors and recombinant plasmids.

E. coli JM83 with the plasmid type	Test bacteria (*E. coli* strains) with plasmid type						
	JM103	*btu*B⁻	pAT11	pAT12	pColE2-AT	pColE2-p9	pMC27
pAT11	+	-	-	-	-	-	+
pAT12	+	-	-	-	-	-	+
pColE2-AT	+	-	-	-	-	-	+
pColE2-p9	+	-	-	-	-	-	+
pMC2 (E9)7	+	-	+	+	+	+	-

+, lysis zone formation; -, no lysis zone formation.

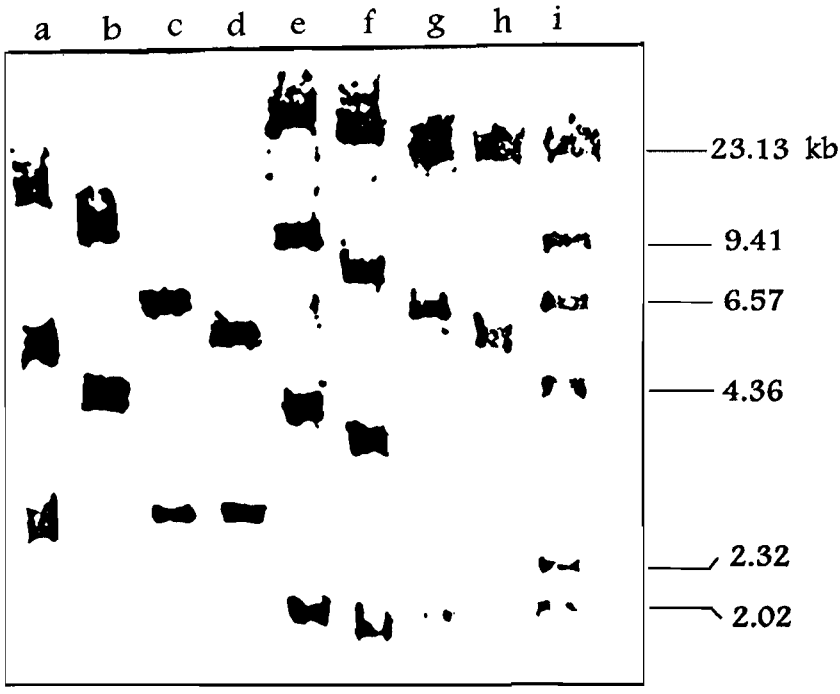

Fig. 1. Gel electrophoresis of plasmid preparations from colicin E2 producers. Lane a and b are undigested pAT11 and pAT12 and lane c and d are *Eco*RI digested pAT12 and pAT11, respectively. Lane e and f are undigested pColE2-P9 and pColE2-AT and lane g and h are *Eco*RI digested pColE2-P9 and pColE2-AT, respectively. Lane i is lambda *Hind*III digested plasmid as a molecular marker.

The undigested plasmid DNAs and the *Eco*RI digested fragments of pColE2-AT and pColE2-P9 exhibited size difference between the two colicin E2 plasmids (Fig. 1). The estimated larger *Eco*RI fragments of 5.8 Kb of pColE2-AT and 6.3 Kb of pColE2-P9 were cloned into *Eco*RI site of the polylinker region of pUC18. These produced recombinant plasmids pAT11 and pAT12, respectively. Transformants of *E.coli* JM83 with the respective recombinant plasmid preparations also displayed the same pattern of properties as the original colicin

E2 encoding plasmids (Table 2). The preparation of these recombinant plasmids confined the essential E colicin operon set of genes of pColE2-AT genes to the larger *Eco*RI fragments. The subsequent localization of the position of these three genes in pAT11 requires molecular techniques, such as, restriction mapping, deletion sub-cloning, and transposon mutagenesis as it was shown by many workers (Chak and James, 1986; Chak *et al.*, 1991).

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